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Inhalation Toxicology of Airborne Particulate Manganese in Rhesus Monkeys

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16. ABSTRACT

Four male and four female rhesus monkeys were exposed to manganese oxide (Mn_3O_4) aerosol at 100 $\mu g/m^3$ in an exposure chamber for periods up to 66 weeks. Three male and three female monkeys were maintained as unexposed controls. Observation and clinical chemistry during the experiment showed minor changes in excretion, and no evidence of toxic effects. Manganese tissue levels showed small increases except in lung (five-fold) and pons (four-fold). No macro- or microscopic pathology was observed. Two rhesus monkeys were exposed to 5 mg/m^3 of the same material for 23 weeks, and observed for 10 months thereafter. Large increases in excretion of manganese were observed in both urine and feces, but no evidence of neurologic or other toxicity was seen. Two groups of 4 and of 14 rats were exposed in the same manner to 100 $\mu g/m^3$ of the same compound for 8 weeks. These experiments were done to examine changes in excretion and tissue levels during and after exposure.

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This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The research on manganese which is described in this report was undertaken because good information on the effects and toxicity of inhaled manganese were not available for prolonged carefully measured exposures. The metal is used as an additive in some distillate fuels, and is currently being considered for broader application. This has caused increased interest in the toxicology of this metal. Attempts are being made by means of new research to correct deficiencies in our knowledge of manganese metabolism and toxicology.

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CONTENTS

Disclaimer ii
Foreword iii
Figures v
Tables vi

1. Summary 1
2. Introduction 2
3. Methods and Materials 3
4. Results 9
5. Discussion 17
6. Conclusions 19

References 67
Pathology Appendix 68

FIGURES

Number		Page
1	Manganese (Mn) generating system	4
2	Manganese delivery system	5
3	Manganese concentration changes during startup and shutdown	10

TABLES

Number		Page
1	Comparison of Air Samples Obtained Inside the Exposure Chamber with Similar Samples Obtained Simultaneously at the Exhaust Duct	20
2	Andersen Sample Studies	21
3	Weekly Mean Concentrations of Mn in Drinking Water	22
4	Monthly Means of Concentration of Mn in Drinking Water	23
5	Body Weights of Monkeys During Exposure to Mn	24
6-19	Clinical Chemistry Profiles	25-38
20-33	Hematology Profiles	39-52
34	Concentration of Mn in Urine of Monkeys Exposed to Airborne Mn	53
35	Concentration of Mn in Feces of Monkeys Exposed to Airborne Mn	54
36	Concentration of Mn in Selected Tissues of Monkeys Exposed to Airborne Mn	55
37	Concentration of Mn in Tissues From Nervous System of Monkeys Exposed to Airborne Mn	57
38	Excretion of Mn in Urine of Rats Exposed to Airborne Mn	58
39	Excretion of Mn in Feces of Rats Exposed to Airborne Mn	59
40	Concentration of Mn in tissues of Rats Exposed to Airborne Mn	60
41	Weekly Mean Concentrations of Mn in Air of High-Level Exposure Chambers	61
42-43	Clinical Chemistry Profiles, High-Level Exposure	62-63
44-45	Hematology Profile, High-Level Exposure	64-65
46	Excretion of Mn by Monkeys Exposed to Airborne Mn at About 5000 $\mu g/m^3$	66

SECTION I

SUMMARY

Male and female rhesus monkeys were exposed to airborne particulate manganese (Mn) at a concentration of 100 ug per cubic meter of air for periods lasting up to 66 weeks. Observations of the animals, including visual observations for any signs of toxicity, and studies of clinical chemistry and hematology did not indicate any effects due to exposure to manganese. Very small increases in the excretion of manganese in the urine and feces were observed signifying, principally, that the burden imposed by the airborne manganese represented only a small fraction of the total daily intake of the metal. In spite of the low level of exposure, a small but significant accumulation of manganese was observed in certain tissues of the animals examined at autopsy. Manganese levels were increased in the lungs, liver, pancreas, kidney, heart muscle, pallium, basal ganglia, cerebellum, and pons. In most cases the degree of increase was two-fold or less, with the exception of the lungs which showed a five-fold increase and the pons which showed a four-fold increase. There were no changes in morphology, either macroscopic or microscopic, which could be attributed to exposure to manganese.

Rats were also exposed to the airborne manganese at 100 ug per cubic meter of air for periods up to eight weeks. Small increases in the excretion of manganese were noted in the animals during exposure which continued for periods up to eight weeks after the animals had been removed from the chamber. Similarly, there was an increase in the concentration of Mn in the lung and brain tissue of exposed rats but, in both cases, there was evidence of a rapid return to normal levels within one week after animals were removed from the exposure chamber.

Two rhesus monkeys were exposed to airborne manganese at about 5 mg per cubic meter of air for a 23 week period of exposure. The principal goal of this study was to determine whether exposure at that level would produce any visible neurologic disorders in the animals. No consistent changes in the clinical chemistry or hematology were observed during the exposure period. The excretion of manganese was greatly increased in both the urine and the feces. Significantly, however, there was no evidence of neurologic disorders or behavioral changes either during the high level exposure, or later after the animals had been removed from the chamber and observed for an additional 10 months.

SECTION I

INTRODUCTION

This is the final report of EPA Contract 68-02-0710, the objective of which was to examine the potential toxicity of airborne particulate manganese when inhaled by rhesus monkeys. Studies were designed to provide definitive data on potential hazards involved in the use of methylcyclopentadienyl manganese tricarbonyl (MMT) when used as a combustion improver in automotive fuels. When used in automotive fuels, the organomanganese compound is burned to produce an oxide of manganese (Mn_3O_4). It was this oxide, in the form of solid particulates, to which the monkeys were exposed. The experiment was designed to provide an almost continuous regimen of exposure of the monkeys to the manganese particulates. The intended level of exposure (100 µg manganese per cubic meter of air) was chosen as a level estimated to be far in excess of that anticipated in ambient air as a result of motor fuel usage and at the same time it was well below the threshold limit value* of manganese. In the main experiment the animals were exposed for one year.

All animals in the study were examined daily for signs of toxicity. Also, periodic clinical examinations of the animals determined the status of their health and well-being. Exposure to manganese was monitored through measurements not only of airborne manganese but also of the manganese content of the diet and drinking water. Excretion of manganese was studied and the concentration of the metal in the animal tissues was determined at autopsy which included gross and histopathologic examination.

Two short ancillary studies of manganese inhalation were also conducted. In one study, data were sought which would yield information on the dynamics of manganese turnover in rats which were exposed to the airborne particulate manganese. In the other study, an effort was made to develop the rhesus monkey as a model for studying acute manganism following exposure at or near the threshold limit value.

* threshold limit value for Mn = 5000 µg/m³, American Conference of Governmental Industrial Hygienists.

SECTION 3

MATERIALS AND METHODS

The system for exposure to manganese particulates was developed at the International Center of Environmental Safety at Holloman AFB, New Mexico. Although similar in basic design to the system used previously by researchers at the Albany Medical College for exposure of animals to airborne particulate lead (1), modifications were incorporated to permit more effective control of the size of the particulate manganese produced by the system. In principle, the system generates manganese oxide particulates through the combustion of vaporized MMT in an air-natural gas flame. The MMT vapor was produced by metering a portion of the air required for combustion through a thermostated container of liquid MMT (Figure 1). Additional metered air was added to the resulting MMT vapors and the mixture was burned in a torch type burner utilizing natural gas as the fuel. The burner was mounted in a combustion chamber located atop a 55-gallon drum holding chamber.

A safety shutoff system was provided to prevent the accumulation of explosive gas mixtures in the holding and exposure chambers as a result of flameout, and also to prevent inadvertent exposure of the animals to unburned MMT. During operation the flame was constantly monitored by a UV detector, and in the event that the flame became extinguished, a high voltage spark ignition system was automatically activated to reignite the flame. This system functioned automatically for approximately 15 seconds, or until the flame was reignited. Failure of the system to reignite the flame resulted in a total shut down of the burner by means of solenoid valves in both the air and natural gas supply lines activated by the UV detector control mechanism. When such a failure occurred it was necessary to reset the system manually before the burner could again be ignited.

The generator system provided control of concentration of manganese particulates in the air, and control of the size of the generated particulates. Adjustment of the concentration of MMT vapors in the combustion mixture maintained control over the final concentration of manganese oxide particulates. The concentration of MMT vapors was varied by altering the temperature of the liquid MMT vessel or the flow rate of the air through the vessel.

Products from the combustion chamber then entered a pair of 55-gallon drum holding chambers aligned in series (Figure 2). These holding chambers served to delay passage of the particulate manganese into the exposure chamber long enough to allow aggregation of the particles, thus yielding particles of a size more likely to be found in the ambient air. A small

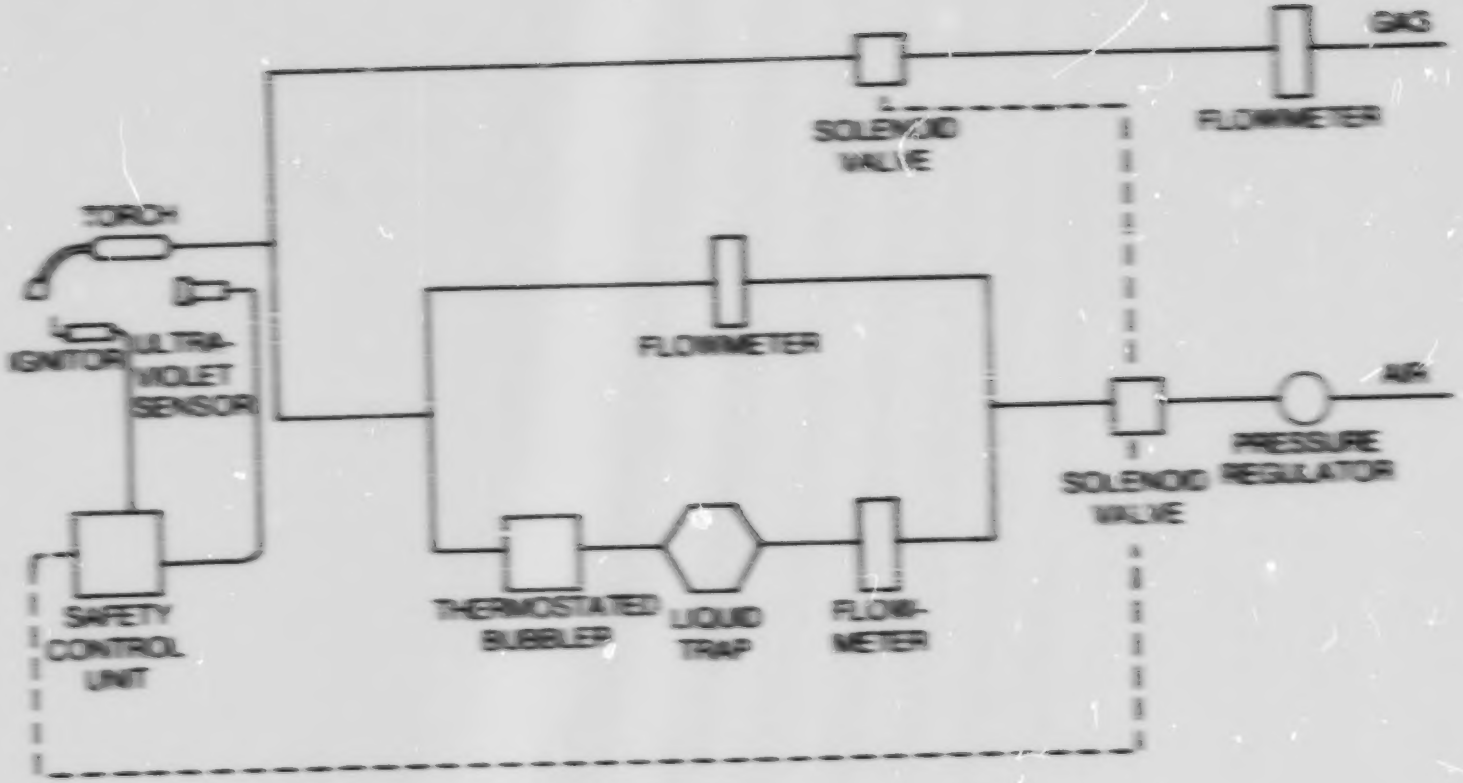

Figure 1. Manganese generating system.

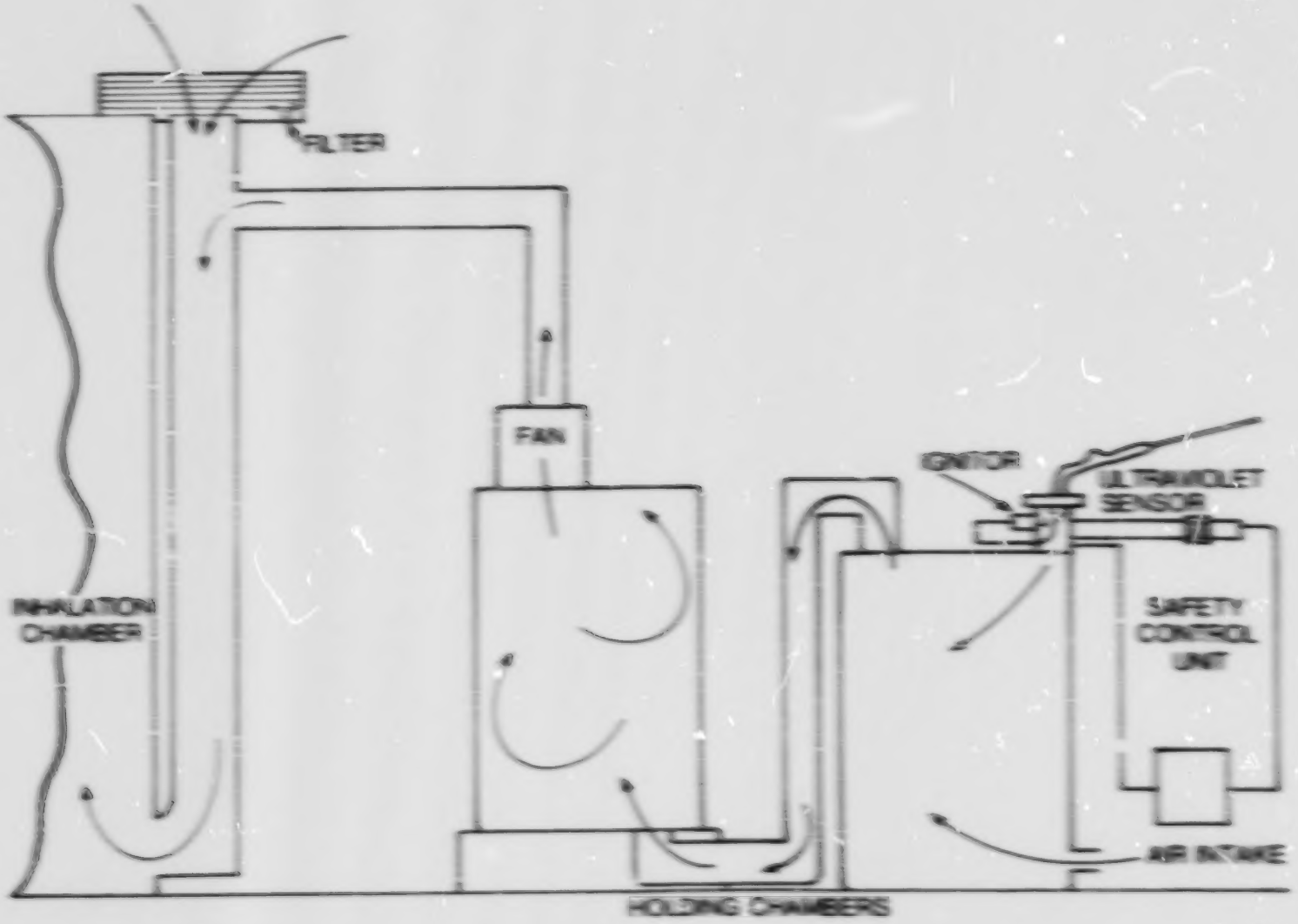

Figure 2. Manganese delivery system.

blower mounted on the second holding chamber extracted its contents at a constant rate and delivered the particulate material to the main exposure chamber.

The exposure chamber itself consisted of a temperature-humidity controlled room, 8 feet wide by 8 feet high and by 12 feet long, modified to permit introduction of the particulate manganese. The effluent from the generator was injected into the intake airduct of the main chamber. Mixing with intake air occurred within the airduct and also within the chamber itself by the action of large circulating fans. An exhaust blower removed air from the chamber at a constant rate and conducted it to the outside of the building.

A second exposure system similar to the one described above and with an identical manganese oxide generating system, but with smaller exposure chambers, was utilized to expose monkeys to a concentration of about 5,000 ug of manganese per cubic meter of air.

Air Sampling

Air samples obtained from the chamber were collected on a daily routine basis to monitor the concentration of manganese in the exposure chamber. The sampling system consisted of a vacuum pump which removed air from the chamber through a particulate filter. A limiting orifice located between the pump and the filter provided control of the airflow rate. The particulate manganese was trapped on a Gelman Type A, 47 mm, glass-fiber filter. The air sampling system ran continuously during periods of exposure. Airflow through the sampling system was frequently calibrated using a spirometer as the standard. Manganese entrapped by the glass-fiber filter was analysed by atomic absorption spectrophotometry. (See below for details of manganese analytical procedures.)

Prior to exposure of animals to the airborne particulate manganese, several studies were conducted to establish the operating parameters of the exposure chamber. These are briefly discussed below:

a. It was thought that a sampling port located in the exhaust duct of the chamber would provide a suitably representative air sample, and to substantiate this, a comparison was made between air samples collected at the duct and similar samples collected by a sampler located within the chamber itself.

b. In order to determine the speed with which the exposure chamber would respond to the daily routine of shutdown and startup, a series of short-term samples were collected during these operations.

c. Size determinations of the particulate manganese in the exposure chamber were made with a modified Andersen Cascade Impactor (2).

d. Measurements of carbon monoxide and carbon dioxide were made periodically in the exposure chamber.

The exposure chamber was routinely operated with the burner on for 23 hours a day, seven days a week. During the one-hour period each day in which the burner was off, all necessary animal care and chamber maintenance was accomplished. Routine air samples consisted of samples collected during the 23 hour period in which the chamber was operating.

Determination of Manganese

Determinations of the manganese content of environmental and biological samples were made using an atomic absorption spectrophotometer (AAS). In all cases the instrument used was a Perkin-Elmer model 303 using conventional air-acetylene flame techniques. The wavelength chosen for analysis was 279.5 nm. All glassware used for determinations of manganese was washed and rinsed thoroughly, and then further soaked in 10% HNO$_3$ and finally rinsed with deionized water. In most cases samples were either ashed dry or digested in acids, a suitable dilution prepared, the solutions aspirated directly into the AAS and compared with aqueous standards. Drinking water samples required preliminary chelation and extraction to prepare solutions of sufficient concentration. Details of the various procedures are outlined below.

Air Samples--

Samples collected on fiber glass filters were placed in 50 ml plastic centrifuge tubes and 25 ml of 10% (v/v) HCl added. The tubes were then placed in a boiling water bath for 10 minutes, cooled, and enough deionized water added to restore the volume to 25 ml. Following centrifugation the samples were ready for aspiration into the AAS.

Drinking Water--

A 50 ml volume of water collected from the automatic watering system of the exposure chamber was placed in 100 ml glass bottles fitted with a cap which utilized a plastic liner. To this was added 20 ml of 0.4 M TRIS buffer, pH 8.0, and the solution mixed well. To this was added 10 ml of a 0.4M freshly prepared solution of ammonium pyrrolidine dithiocarbamate and the mixture again shaken. Finally 10 ml of water-saturated methyl isobutyl ketone was added, the mixture shaken for one minute, the layers allowed to separate and the organic phase transferred to a clean glass tube. The organic phase was then aspirated into the AAS and compared with standards similarly chelated and extracted.

Animal Diets--

Purina Monkey Chow biscuits were ground to a fine powder and 1.0 gram accurately weighed into a porcelain crucible. The sample was then ashed overnight at 550°C. Four drops of concentrated HNO$_3$ and 10 drops of concentrated HCl was then added to the cooled crucible which was then heated on a hot plate. A minimal volume of deionized water was used to transfer the acid digest to a 25 ml volumetric flask. The acid/water transferring procedure was repeated two more times, the flasks brought to volume with deionized water, mixed well, and the samples aspiration into the AAS.

Urine Samples--

Urine was collected from each animal for 24 hours, and the volume determined. To each sample, 10 ml of glacial acetic acid was then added,

acid the samples stored in plastic under refrigeration until ready for analysis. Approximately 5 ml aliquots were filtered into a clean glass tube, aspirated into the AAS and compared with aqueous standards.

Fecal Samples--

Feces were collected from each animal for 24 hours, weighed and dried, and the dry weight determined. A 0.25g portion was weighed into a porcelain crucible, ashed overnight at 550°C and then transferred with acid and dionized water as for the diet samples (see above).

Tissue Samples--

Accurately weighed portions of tissue in the range of 2 to 4 grams obtained at autopsy were placed in porcelain crucibles. The samples were dried at 100°C for 3 hours, and then ashed at 550°C overnight. The residue was transferred 2 or 3 times using 2 drops of concentrated HCl, 5 drops of concentrated HNO_3 and sufficient deionized water for a final volume of 2 ml or 10 ml depending on the tissue. The aqueous solutions were then aspirated into the AAS.

Animal Studies

The animal exposures were initiated with a study of eight rhesus monkeys (four male and four female *Macaca mulatta*) with three additional animals of each sex serving as nonexposed controls. The animals were allowed free access to food and water throughout the experiment. Body weights of the animals were determined at monthly intervals. Each animal was also observed daily for signs of toxicity or for any other signs of exposure to airborne manganese.

Periodically, a series of clinical chemistry and hematology studies were performed on the animals. Hematology studies included total erythrocyte and leucocyte counts, packed cell volume and hemoglobin concentration. Serum chemistry studies included determination of sodium, potassium, chloride, calcium, creatinine, urea nitrogen, phosporous, bilirubin, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, lactate dehydrogenase, and total cholesterol.

Definitive manganese balance studies were not attempted, but the diet and excreta were monitored on a routine basis for their manganese content.

SECTION 4

RESULTS

EXPOSURE CONDITIONS

Results of the study comparing air sampling at the exhaust duct and within the chamber itself are shown in Table 1. Although the samples collected at the duct were slightly lower than those collected within the chamber, the differences were very small and within the limits of experimental error. This established the validity of using the exhaust-duct sampling port for routine measurements. A similar series of tests showed that the concentration determined by a single 24-hour sample was very close to the average of several one-hour samples collected during the same 24-hour period. This established the reliability of the 24-hour sample which also had the advantage of yielding integrated results over the entire daily operating period.

Buildup of the proper concentration of manganese oxide in the chamber during the daily routine of startup is shown in Figure 3. The results of this study demonstrated that within less than 30 minutes after the burner was ignited the proper manganese concentration was reached. Within a similar period the chamber contents were exhausted of manganese after the burner was turned off. This is also shown in Figure 3.

The results of our studies to determine the size of the generated manganese oxide particulates using the modified Andersen Cascade Impactor are shown in Table 2. In this table, the results of these studies are compared with the lead particulate measured during our previous human studies (1) and with lead particulate collected in ambient air near a roadway. These data demonstrate the effectiveness of the 55-gallon-drum holding chambers in increasing the size of the generated particulates.

Periodic measurements of carbon dioxide and carbon monoxide in the exposure chambers established that the levels of these materials were well within acceptable ranges. The concentration of carbon dioxide never exceeded 0.15 and the concentration of carbon monoxide was estimated to be about one part per million.

The weekly means of concentrations of manganese in the air of the exposure chamber is shown in Table 3. Each mean shown is the average of the seven daily determinations. Also shown in the table is the overall mean for 52 weeks and, since some animals were exposed for an additional 14 weeks, this 66 week mean is also shown. These data demonstrate that the intended concentration of 10 mg of manganese per cubic meter if air was very nearly achieved although there were some weekly fluctuations.

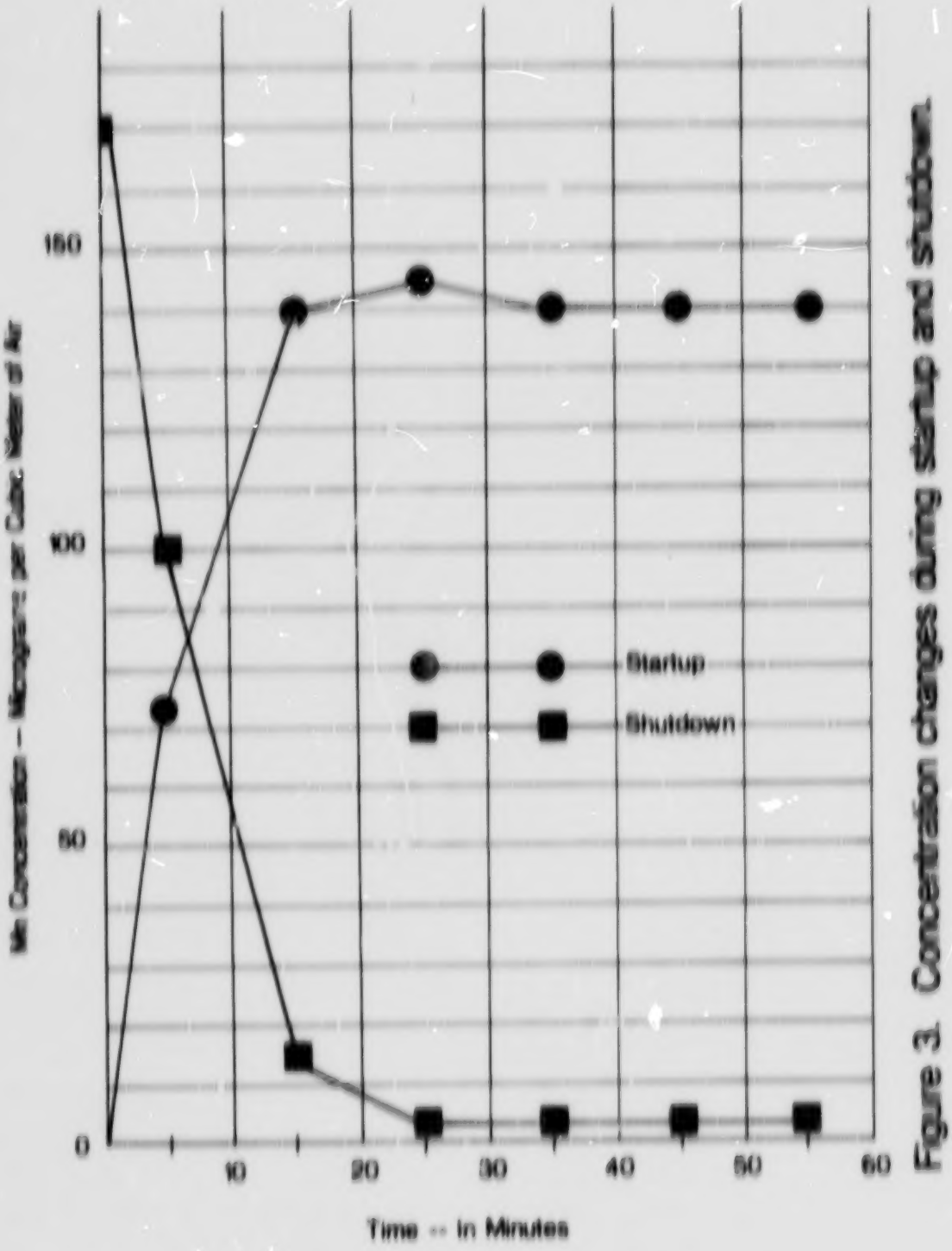

Figure 3. Concentration changes during startup and shutdown.

The diet and drinking water of the animals also contributed, to some extent, to the exposure of manganese. The average concentration of manganese in the Purina Monkey Chow was 47 µg per gram of diet. The concentration of manganese in drinking water determined at monthly intervals is shown in Table 4. During the study, the weekly average concentrations of manganese varied only from 0.017 µg to 0.033 µg per ml of water.

Clinical Evaluations

Throughout the investigation, all of the animals were observed on a daily basis for any signs of toxicity. At all times during the study the animals were normal in appearance and did not display any behavior or any other visual manifestations of toxicity attributable to exposure to manganese at 100 µg per cubic meter of air. Growth was monitored by periodic determinations of body weights, and these data are shown in Table 5. Body weights and body weight gains were normal and did not show any effect of exposure to manganese.

Blood samples were obtained on a periodic basis for clinical biochemistry studies. Parameters measured were selected to show any changes in electrolyte balance, kidney status, liver status, and general metabolic status. Profiles for each animal are shown in Table 6 through 19. There was no evidence in any of the animals exposed to 100 µg of manganese per cubic meter of air which would indicate an alteration of status in any of the parameters chosen for study.

In a similar fashion, hematology profiles of each animal were determined periodically throughout the study. These data are shown in Tables 20 through 33. Again, there was no evidence of an effect on the parameters chosen for investigation.

Excretion of Manganese

At intervals during the investigation, the excretion of manganese in the urine and feces of the animals exposed to airborne manganese was evaluated. In order to obtain suitable samples, it was necessary to remove the animals from the exposure chamber during a 24-hour period for collection of the samples. This was necessary to avoid contamination of the urine and feces samples collected in metabolism cages by the airborne manganese present in the exposure chamber. The entire volume of urine and weight of feces samples collected from each animal was recorded during these 24-hour periods.

Urinary excretion of manganese by the monkeys is shown in Table 34. These data express excretion as the total amount of managanese excreted in a 24-hour period. It is noteworthy that in all cases the amount of manganese excreted was very small regardless of the condition or period of exposure. The amount of manganese excreted under any of these conditions was always less than 100 µg in the 24-hour period. Considerable variations were also observed in the individual rates of excretion and also in the amount of manganese excreted by any one animal at different times, regardless of the condition of exposure. For example, in the control groups the amount of manganese excreted ranged from less than 1 µg to as high as 41 µg for one

particular animal. Similarly, in the exposed group the elimination of manganese ranged from 1 to 77 µg excreted in a 24-hour period. Although it is difficult to make generalizations from the data, there was a trend to greater excretion of manganese during exposure than either during preexposure or among control animals. This was not universally observed, however, and in at least one instance the rate of excretion was greater during the baseline period than after 8 or 12 months of exposure. The significant decrease in the rate of excretion of manganese among the control animals after the 12th month of the experiment is not understood. The analyses were performed by the same technical staff using the same procedures and equipment utilized throughout the study.

The excretion of manganese in the feces of the animals is shown in Table 35. Based on the amount of manganese excreted in a 24-hour period, the rate of excretion of the metal in the feces is in the range of three orders of magnitude greater than in the urine samples obtained at the same time. Note that the data in Table 35 are expressed as milligrams excreted in a 24-hour period. Here again, however, there are variations in the excretion rate of manganese from individual to individual and within the same individual from time to time regardless of condition of exposure or duration of exposure to manganese. Thus, at any one time, for example the four-month period of exposure, the rate of excretion of manganese is greater in the control group than in the exposed group. In fact, examination of the data throughout the study indicated a somewhat greater level of excretion of manganese by the control animals than by the exposed animals.

Studies by other investigators (4, 5, 6) have shown that excretion of manganese is principally through the gastrointestinal tract. This is substantiated in the present study in which the highest concentration of the metal is found in the feces. It is not possible to speculate on the disposition of the small amount of manganese to which the animals were exposed in the chamber experiment. If it is accepted that the respiratory minute volume for rhesus monkeys is about 0.86 liters per minute (7), then one can compute that the animals were exposed to a little more than 100 µg manganese in a 24-hour period in the chamber containing about 100 µg of manganese per cubic meter of air. Even if one assumes 100% absorption of the manganese by the lungs, which is unlikely, then it can be seen that the amount of manganese to which the animals were exposed in the air is only a very small fraction of a total amount of manganese excreted by the animals in a 24-hour period. These data indicate that, even among the animals in the exposure chamber, the greatest source of exposure to manganese was not the air but rather some other source. Examination of the diet indicates this as that source. If the dietary concentration is on the order of 47 µg of manganese per gram of solid diet as indicated by our studies, then a animal consuming approximately 100 g of the dry diet would consume between 4 and 5 mg of manganese in a 24-hour period. This is consistent with the rate of excretion of manganese that we observed in this experiment.

Accumulation of Manganese

Although exposure to airborne manganese could account for only a small fraction of the total intake of manganese in a 24-hour period, this is not to say that distribution of metal within tissues of the animals was not altered. At autopsy a number of tissues obtained from the animals were examined for their manganese content. The control animals for this phase of the study, although not the same as those used in other phases of the study, were, nonetheless, in our colony for the same period of time as the animals in the main manganese study, and were housed and otherwise maintained under identical conditions to the controls used in the excretion studies and clinical investigations. They were, in fact, housed in the same room in our animal quarters. The concentration of manganese found in the animals following different conditions of exposure to the metal in the air is shown on Tables 16 and 17. Concentrations in the lung, liver, pancreas, spleen, kidney, bone, adrenal gland, heart muscle, and skeletal muscle are shown in Table 16. Where sufficient numbers were available, as was the case for those animals sacrificed after 12 months of exposure, it was possible to compare the means using the Students t-test. This indicated that levels of manganese were slightly but significantly increased in the lungs, liver, pancreas, kidney, and heart muscle. Among those tissues showing a significant increase, the degree of increase was by a factor of two or less, with the exception of the lung in which the concentration of manganese was on the order of five times the level found in the nonexposed animals. The number of animals sacrificed at periods other than 12 months was not sufficient to permit similar statistical comparisons. However, the trends reflected those found in the animals exposed for 12 months.

It should be noted here that a considerable effort was made to monitor levels of manganese in blood samples obtained from the monkeys. Although we were not able to develop a procedure capable of yielding consistantly acceptable data, we were able to determine that manganese levels in blood samples of the rhesus monkeys was of the order of about 0.06 to 0.10 ug/g when measured in either whole blood or serum. Comparable levels were encountered in both control and exposed animals.

We were also able to measure the concentration of manganese in selected tissues from the nervous system. Manganese was measured in the pallium, basal ganglia, cerebellum, pons, and medulla. Some increases in manganese content was also observed here among the animals exposed to airborne manganese, and we were able to utilize the same statistical treatment on the animals exposed for 12 months. The manganese concentration was significantly greater in the pallium, basal ganglia, cerebellum, and pons. The degree of difference was again about two-fold with the exception of the pons which had four times the amount of manganese in the exposed animals compared with the control animals.

The concentrations of manganese observed in the rhesus monkeys in the present study are remarkably similar to concentrations of manganese found in similar tissues from other animal species (8). A notable exception to this is the level which we found in adrenal tissue. In the monkey, even among the control animals, the amount of the metal in the adrenal seems to be about 10 times that of the amount found in man, and about twice the amount found in

rabbits. Although manganese was significantly increased in some tissues of the exposed animals, the degree of this increase was not extremely large nor was the biological significance readily apparent.

Morphological and Histopathological Studies

Sacrificed animals were subjected to a thorough gross and microscopic examination. With one exception all of the changes observed constituted nonspecific normal pathological observations of rhesus monkeys in our colony. The only exception to this was the observation of monocytic and lymphocytic infiltration of the myocardium. The degree in each case was only minimal and the change was observed in only three of the exposed monkeys which were autopsied. Although the changes were found only in animals from the exposure chamber, there was insufficient evidence to implicate manganese as the causative agent.

The complete pathology report is included as an Appendix.

Exposure of Rats to Airborne Manganese

Following our observation of some increase in certain tissue levels of manganese in the animal exposed for 6 months, we conducted a short experiment with rats to substantiate this observation. We also hoped to obtain additional data on the dynamics of excretion of manganese. Two groups of animals were studied. In the first group, the excretion of manganese in the urine and feces of four rats was examined. These animals were then placed in the chamber, and subsequently the excretion was measured after one, four, and eight weeks of exposure. When eight weeks of exposure was completed, the animals were then removed from the chamber and the excretion of manganese in the urine and feces again was measured after one, four, and eight weeks following the exposure period. The second group of 14 animals was used to study the concentration of manganese in tissues. The concentration of manganese in liver, kidney, lungs and whole brain was measured in two animals prior to the exposure period. Animals were then placed in the chamber and pairs of animals killed after one, four and eight weeks of exposure. The remaining animals were then removed from the exposure chamber and pairs killed after one, four, and eight weeks postexposure.

The excretion of manganese in the urine of the rats is shown in Table 38. In this table the data are expressed both as ug of manganese per milliliter of urine and as ug of manganese excreted in a 24-hour period. The only generalization that can be made from these data is that the excretion of manganese apparently increased after the animals were placed in the chamber and that this excretion at an elevated rate appeared to continue even after the animals were removed from the chamber. A similar increase in the rate of excretion of manganese following exposure was demonstrated in the fecal data shown in Table 39. Again the increased rate of excretion apparently continued even eight weeks after the animals were removed from the exposure chamber.

The concentration of manganese found in the tissues of rats exposed to airborne manganese is shown in Table 40. There were definite increases observed in the lung and brain of the exposed animals, but the liver and kidney data were inconclusive. In both the lung and the brain there was evidence of a rapid return to normal levels within one week after the animals were moved from the chamber.

Because of the low level of exposure, the data obtained with these studies in rats were insufficient to permit conclusions regarding the turnover rate of manganese in the body. The results obtained were not inconsistent with published results using radioactive manganese (9). These have shown disappearance of manganese from the body with two exponential components, one with half-time of 19 days and one with half-time of four days.

High-Level Exposure

Neurologic manifestations of manganese toxicity have been described in rhesus monkeys exposed to very high levels of the metal (10). Epidemiologic studies in humans also exposed to very high levels of manganese dust suggests a neurologic disorder with a Parkinson-like syndrome (11). The threshold limit value for an eight-hour exposure has been established at five mg per cubic meter of air. We conducted a study with two rhesus monkeys exposed to manganese at the threshold limit value except that the animals were exposed for 23-hours per day rather than eight hours. It was the intent of this study to determine whether exposure at this level would also produce any visible neurologic disorders in rhesus monkeys.

The system for generating the high level of manganese was identical to that previously described for the study at 100 ug per cubic meter of air. The principal difference in the two systems was that, in the case of the high level of exposure, the chambers were fed directly from the generator without the addition of outside air. Each of the two chambers housed one rhesus monkey and daily measurements were made to establish the concentration of manganese within each chamber. These data are shown in Table 41. The mean concentration of manganese over the 23-week exposure period was for 4.86 mg per cubic meter of air in one chamber and 5.10 mg per cubic meter of air in the second chamber. The animals were observed daily for any signs of toxicity, and during the exposure phase of the study, periodic clinical chemistry and hematology samples were obtained. Following the exposure period of the study the animals were not sacrificed but were removed from the chamber and observed for an additional 10 months.

Clinical chemistry data for the two animals are shown in Tables 42 and 43. The only changes observed were small, inconsistent increases in the level in inorganic phosphate in the sera. Significantly, there were no increases in either of the transaminases or LDH which might have signified tissue damage. Hematology data shown in Tables 44 and 45 do not show any effects attributable to the high level of manganese.

Excretion of manganese by the two monkeys is shown in Table 46. These data demonstrated significant increases in the excretion of the metal both by the urinary and by the gastrointestinal route. Of particular interest is

the very significant increase in urinary excretion signifying the extent to which the kidney was involved in removal of manganese from the body following a very high level of exposure. During exposure, the excretion rates were increased by one or two orders of magnitude over excretion rates during the preexposure period.

No evidence of neurological disorders was observed in the animals during exposure to the high level of airborne manganese. Neither did any such symptoms become evident during the 10-month postexposure period.

SECTION 5

DISCUSSION

The goal of these investigations was to provide safety evaluation data on airborne manganese particulates which might be encountered in ambient air resulting from the use of MMT as a combustion improver in automotive fuels. In achieving this goal, monkeys were chronically exposed to the combustion products of MMT at a level of about 100 ug per cubic meter of air in an environmentally controlled exposure chamber. The level of exposure was far in excess of the current levels of lead measured in the atmosphere over cities in the United States. Although the manganese particulates generated by the exposure system were not expected to be identical to manganese particulates counted in ambient air (as a result of the use of MMT in motor fuels), the size of particles was somewhat similar to the size of particulate lead found in the ambient air. Efforts were made to match the size of lead particulates found in the air but the size of the manganese particulates was somewhat smaller. The observed differences in size were small, however, and the laboratory system can be considered to be a suitable model for ambient conditions.

Similar statements probably could not be made with regard to the composition of the manganese oxide particulates from automobile exhaust systems. We considered that our exposure systems generated a relatively pure form of manganese oxide. The manganese oxide found in automotive exhaust fumes would likely be associated with other particulate matter formed in the exhaust fumes and with other components of the atmosphere as well. These differences could be expected to have an effect on the amount of manganese absorbed from the particulates by the lungs of the animals.

Clinical data and visual observations of the animals showed that they tolerated the exposures well. In fact, these data and observations did not divulge any effects which could be attributed to manganese. This was not unexpected since the additional body burden of manganese imposed by inhalation of the particulates did not contribute significantly to the total of manganese taken in daily by the animals. It was necessary to seek out subtle changes which reflected respiratory exposure to the airborne manganese although not necessarily indicating a toxic effect on the animals.

Small but statistically significant differences were found in the concentration of manganese in selected tissues of the exposed animals. The largest increase, as would be expected, was found in the lungs of the animals. Lesser increases were found in the liver, pancreas, kidney, heart muscle and certain tissues of the nervous system. Increases in manganese content were not correlated with any functional or morphologic effects on tissues or organs.

The only large changes in the rate of the manganese excretion observed in these studies were those encountered in the animals exposed to 5 mg of manganese per cubic meter of air. This is not surprising since the routes and hence the daily intake of manganese by these animals was largely increased over normal intake per day. Airborne exposure of as much as 5 mg of manganese per day produced levels of manganese in the chamber which were extremely high, it is not unlikely that additional manganese intake occurred through the alimentary tract from manganese particulates settling on food, on the animals' hair, and on other surfaces in the chamber. Thus, even grooming behavior could increase the manganese intake by these animals. All of the excretion data indicated that the largest fraction of manganese was excreted in the feces. It is significant, however, that the excretion rate of manganese in the urine of animals exposed 5 mg/m^3 of airborne manganese was very largely increased. This points out the significance of the role played by the kidneys in eliminating manganese from the body during periods of high level exposure.

SECTION 6

CONCLUSIONS

Monkeys Exposed to 100 ug of Manganese per Cubic Meter of Air

(1) Chronic exposure to airborne manganese does not induce any change in appearance or behavior.

(2) Chronic exposure is not reflected in any changes in clinical chemistry profiles or hematology profiles.

(3) Airborne manganese at 100 ug cubic meter of air does not contribute significantly to the total intake of manganese although excretion rates are slightly increased.

(4) Small but significant increases of manganese in lungs, liver, pancreas, kidney, heart muscle, pallium, basal ganglia, cerebellum and pons result from exposure to 100 ug per cubic meter of air.

(5) No gross or microscopic changes were observed in any of the tissues or organs which could be attributed to manganese.

Rats Exposed to 100 ug of Manganese per Cubic Meter of Air

(1) Eight weeks of exposure causes small increases in the excretion of manganese which persist even eight weeks following exposure of the animals.

(2) Concentrations of manganese in the lungs and brain increases following exposure for eight weeks but decrease to normal levels within one week following exposure.

Exposure of Monkeys to Airborne Manganese at 5 mg per Cubic Meter of Air

(1) No significant changes in chemistry profiles or hematology are observed in animals exposed for 23 weeks.

(2) Excretion of manganese in the urine and feces is very greatly increased.

(3) No neurological disorders occur in the animals during exposure nor for at least 10 months following the exposure period.

TABLE 1. COMPARISON OF AIR SAMPLES OBTAINED INSIDE THE EXPOSURE CHAMBER WITH SIMILAR SAMPLES OBTAINED SIMULTANEOUSLY AT THE EXHAUST DUCT*

	Inside chamber	Exhaust chamber
	98	99
	104	102
	104	99
	104	98
	96	93
	98	94
Mean	101	98

*Data are expressed as micrograms of manganese per cubic meter of air ($\mu gMn/m^3$ air).

TABLE 2. ANDERSEN SAMPLE STUDIES

Stage	50% Cutoff, microns	Lead near road 400 ft.	20 ft.	Lead, human chamber	Manganese, animal chamber*
1	4	7.3	6.4	1.6	1.1
2	2.6	6.5	7.2	1.5	3.5
3	1.5	7.3	7.2	1.9	2.6
4	0.9	6.9	6.0	2.8	4.3
5	0.4	17.6	10.4	7.5	9.0
6	0.18	20.2	15.6	13.9	23.0
7	<0.18	34.9	46.3	70.8	58.3

* 55 gallon drum chamber

TABLE 3. WEEKLY MEAN CONCENTRATIONS OF Mn IN AIR OF EXPOSURE CHAMBER[a]

Week	Conc	Week	Conc	Week	Conc	Week	Conc
1	92	18	80	35	86	53	123
2	85	19	95	36	102	54	105
3	171	20	100	37	111	55	105
4	103	21	104	38	116	56	110
5	71	22	110	39	97	57	82
6	57	23	98	40	100	58	76
7	90	24	103	41	90	59	99
8	63	25	146	42	110	60	68
9	107	26	131	43	103	61	84
10	116	27	109	44	120	62	77
11	94	28	96	45	95	63	70
12	103	29	97	46	94	64	76
13	106	30	104	47	111	65	117
14	94	31	93	48	125	66	139
15	109	32	97	49	102		101 ± 19[2]
16	104	33	108	50	117		
17	113	34	105	51	100		
				52	126		
					103 ± 18[1]		

[a]Each listed value is the mean of seven daily samples. Data are expressed as ugMn/m^3 air.

[1]Mean and standard deviation of weeks 1-52

[2]Mean and standard deviation of weeks 1-66

TABLE 4. MONTHLY MEANS OF CONCENTRATION OF MANGANESE IN DRINKING WATER

Month	Concentration	Month	Concentration
1	0.028	9	0.031
2	0.033	10	0.033
3	0.029	11	0.021
4	0.030	12	0.020
5	0.026	13	0.023
6	0.024	14	0.018
7	0.031	15	0.017
8	0.029		

*Values are means of weekly determinations and are expressed as ug/ml water.

TABLE 5. BODY WEIGHTS OF MONKEYS DURING EXPOSURE TO MANGANESE EXPRESSED IN KILOGRAMS

Animal number	Baseline weight	1 mo	2 mo	3 mo	4 mo	5 mo	6 mo	7 mo	8 mo	9 mo	10 mo	11 mo	12 mo
CONTROL													
17[illegible]P	4.00	5.00	4.50	5.00	5.00	6.35	6.25	5.30	6.80	7.00	7.10	7.50	6.50
17[illegible]P	4.50	—	4.50	5.00	5.75	5.80	6.00	6.30	6.90	7.50	8.00	8.30	8.60
18[illegible]P	2.75	3.50	3.25	3.50	4.00	4.30	–[1]						
1403F	—	5.00	4.75	5.25	5.75	6.30	6.00	5.70	6.20	6.10	5.90	5.50	5.90
1540F	4.25	5.00	4.50	4.75	5.25	6.40	6.35	5.80	6.10	6.20	5.70	6.40	6.80
1516F	4.25	4.75	4.50	4.75	4.50	4.60	—	4.10	4.60	4.50	4.70	4.50	4.30
EXPOSED													
1543P	4.25	4.75	5.25	—	6.00	6.50	–[1]						
1665P	3.00	3.50	4.00	—	4.50	4.30	5.60	4.40	4.70	4.90	5.10	5.30	5.70
1670P	2.75	3.50	3.50	—	4.00	4.10	4.20	4.40	4.50	4.80	4.90	5.20	—
1677P	3.75	4.50	4.75	—	5.25	5.50	4.90	5.70	6.00	6.10	6.20	6.20	—
1913F	4.75	4.50	5.00	—	4.75	6.00	6.00	5.10	5.90	5.90	5.90	5.80	—
1919F	4.25	4.50	4.75	—	5.00	5.20	5.30	5.20	5.30	5.50	5.20	5.70	5.50
1920F	4.50	5.00	5.25	—	5.50	5.50	5.50	5.30	5.00	5.10	5.40	5.50	6.00
1922F	3.75	4.50	4.50	—	4.75	—	4.50	4.50	4.50	4.50	5.70	4.70	—

[1]Sacrificed after 6 months.

TABLE 6. CLINICAL CHEMISTRY PROFILE OF ANIMAL NUMBER 1403 (FEMALE, CONTROL)

	Na^{+} (mEq/l)	K^{+} (mEq/l)	Cl^{-} (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	Bili. (mg%)	SGOT (U/ml)	SGPT (U/ml)	LDH (Sigma U)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	143	4.3	104	5.3	0.88	23	0.10	41.0	34.0	1560	179	5.00
Baseline II	145	3.9	107	4.9	1.00	20	0.30	37.0	22.0	420	95	-
1 Month	140	4.7	109	5.0	0.90	25	0.30	38.0	21.0	720	132	-
2 Months	142	4.3	105	5.0	0.90	24	0.40	40.0	13.0	550	109	4.10
4 Months	144	5.0	110	5.1	0.80	10	0.70	34.0	14.0	740	130	4.40
6 Months	150	4.1	103	5.2	0.90	19	0.35	27.0	19.0	350	100	3.50
8 Months	136	3.6	107	4.7	0.90	10	0.38	36.0	11.0	660	109	4.20
12 Months	142	3.6	106	4.8	1.10	30	0.10	31.0	12.0	770	110	4.30

Note: There was a trace of hemolysis at one month only.

TABLE 7. CLIMATIC CHARACTERISTICS OF SELECTED AIRPORTS (STANDARD, CONTINUED)

Airport	Elev. (ft)	Mean Daily Max. Temp. (°F)	Mean Daily Min. Temp. (°F)	Mean Annual Temp. (°F)	Mean Annual Precip. (in)	Mean Annual Snowfall (in)	Mean Annual Heating Degree Days (°F)	Mean Annual Cooling Degree Days (°F)	Mean Annual Wind Speed (mph)	Prevailing Wind Direction
Region II										
1 Albany	285	59.1	37.5	48.3	39.9	64.3	5,000	600	9.2	NW
2 Buffalo	705	54.2	36.1	45.2	36.1	93.1	5,600	400	12.5	SW
3 Elmira	1,020	57.2	36.1	46.7	36.1	64.3	5,300	500	8.9	W
4 Islip	99	59.1	41.2	50.2	43.5	28.0	4,800	600	10.3	NW
5 New York	132	62.3	47.1	54.7	43.5	25.5	4,300	1,000	10.3	NW
6 Rochester	559	56.2	36.1	46.2	32.1	94.1	5,600	400	10.3	W
7 Syracuse	421	57.2	36.1	46.7	36.1	114.3	5,400	500	9.2	W
8 Utica	1,004	56.2	34.1	45.2	36.1	114.3	5,600	400	8.9	W
9 Watertown	325	57.2	36.1	46.7	36.1	114.3	5,400	500	9.2	W
Region III										
1 Allentown	384	62.3	41.2	51.8	43.5	28.0	4,500	800	9.2	NW
2 Harrisburg	310	64.4	43.3	53.9	43.5	28.0	4,300	900	8.9	NW
3 Philadelphia	30	64.4	47.1	55.8	43.5	25.5	4,000	1,000	10.3	NW
4 Pittsburgh	1,203	57.2	36.1	46.7	36.1	64.3	5,300	500	9.2	W
5 Reading	344	64.4	43.3	53.9	43.5	28.0	4,300	900	8.9	NW
6 Scranton	1,000	59.1	37.5	48.3	39.9	64.3	5,000	600	9.2	NW
7 Wilkes-Barre	1,000	59.1	37.5	48.3	39.9	64.3	5,000	600	9.2	NW
8 Williamsport	525	62.3	41.2	51.8	43.5	28.0	4,500	800	9.2	NW

Note: There are a number of Region III airports not listed.

| | | |

TABLE 6. CLINICAL CHEMISTRY PROFILES OF ANIMAL NUMBER 1176 (MALE, CONTROL)

Day	Na	K	Cl	CO_2	Anion Gap	BUN	Creat	Glucose	Ca	Phos	T.Prot	Alb	Glob	A/G	T.Bili	Alk Phos	SGOT	SGPT	LDH	CPK
Baseline 1	150	4.5	107	27	16	17	0.7	96	10.1	6.9	6.9	4.3	2.6	1.7	0.2	109	87	51	1,168	1,168
Baseline 2	150	4.5	107	27	16	17	0.7	96	10.1	6.9	6.9	4.3	2.6	1.7	0.2	109	87	51	1,168	1,168
Week 1	150	4.3	107	27	16	17	0.7	96	10.1	6.9	6.9	4.3	2.6	1.7	0.2	109	87	51	1,168	1,168
Week 2	150	4.3	107	27	16	17	0.7	96	10.1	6.9	6.9	4.3	2.6	1.7	0.2	109	87	51	1,168	1,168
Week 4	150	4.3	107	27	16	17	0.7	96	10.1	6.9	6.9	4.3	2.6	1.7	0.2	109	87	51	1,168	1,168
Week 8	150	4.3	107	27	16	17	0.7	96	10.1	6.9	6.9	4.3	2.6	1.7	0.2	109	87	51	1,168	1,168
Week 12	150	4.3	107	27	16	17	0.7	96	10.1	6.9	6.9	4.3	2.6	1.7	0.2	109	87	51	1,168	1,168

Values = "Same" as in preceding analysis.

TABLE 10. CLINICAL CHEMISTRY PROFILE OF ANIMAL NUMBER 1867 (MALE, CONTROL)

	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	Bili. (mg%)	SGOT (U/ml)	SGPT (U/ml)	LDH (Sigma U)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	138	5.5	107	5.4	0.75	35	0.30	39.0	27.0	1100	209	4.60
Baseline II												
1 Month	136	3.7	104	5.0	0.90	14	0.30	46.0	27.0	690	202	-
2 Months	137	3.8	99	5.0	0.80	17	0.50	43.0	16.0	610	168	5.50
4 Months	143	4.5	105	5.0	0.75	15	0.40	32.0	16.5	530	204	5.70
6 Months	149	4.9	100	5.3	0.85	18	0.50	32.0	19.0	540	150	6.00

Note: There was no evidence of hemolysis.

TABLE 11. CLINICAL CHEMISTRY PROFILE OF ANIMAL NUMBER 1916 (FEMALE, CONTROL)

	Na^{+} (mEq/l)	K^{+} (mEq/l)	Cl^{-} (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	Bili. (mg%)	SGOT (U/ml)	SGPT (U/ml)	LDH (Sigma U)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	166	6.6	102	6.3	1.10	16	q.n.s.[1]	41.0	41.0	1070	191	3.30
Baseline II	151	4.5	99	5.2	1.00	15	0.05	28.0	39.0	700	155	-
1 Month	136	4.4	104	5.4	0.98	16	0.35	37.0	29.0	940	206	-
2 Months	140	4.0	103	4.8	0.85	15	0.38	28.0	29.0	520	124	-
4 Months	142	4.8	106	5.0	0.70	19	0.45	28.0	33.0	610	174	3.45
6 Months	143	4.5	96	5.2	0.90	23	0.55	33.0	46.0	840	125	2.40
8 Months	142	3.6	100	5.0	0.80	29	0.55	25.0	21.0	500	135	2.20
12 Months	146	3.8	105	4.7	0.91	23	0.20	40.0	32.0	1240	143	3.70

[1]q.n.s. = quantity not sufficient.

Note: There was a trace of hemolysis at one month only.

TABLE 12. CLINICAL CHEMISTRY PROFILE OF ANIMAL NUMBER 1549 (MALE, EXPOSED)

	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	Bili. (mg%)	SGOT (U/ml)	SGPT (U/ml)	LDH (Sigma U)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	148	4.4	97	5.4	1.10	15	0.35	33.0	25.0	330	169	4.35
Baseline II	146	4.2	103	5.5	0.90	19	0.14	41.0	39.0	400	200	3.10
1 Month	140	4.9	108	5.1	0.50	12	0.20	34.0	18.0	530	167	-
2 Months	144	4.7	104	5.3	0.95	14	0.46	35.0	16.0	570	183	6.80
4 Months	147	5.3	107	5.3	0.80	15	0.52	26.0	16.0	420	191	4.80
6 Months	150	4.5	99	5.4	0.95	18	0.42	30.0	19.0	520	167	5.90

Note: There was no evidence of hemolysis.

TABLE 12. CLINICAL CHEMISTRY PROFILE OF ANIMAL NUMBER 1869 (MALE, EXPOSED)

	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	Bilir. (mg%)	SGOT (u/ml)	SGPT (u/ml)	LDH (Sigma u)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	158	5.8	111	5.4	0.80	30	q.n.s.[1]	44.0	41.0	700	162	4.20
Baseline II	157	5.6	105	5.3	1.10	15	0.05	38.0	34.0	1140	205	-
1 Month	141	4.0	105	4.9	0.80	19	0.25	38.0	31.0	820	195	-
2 Months	149	4.9	106	5.1	0.96	18	0.55	36.0	21.0	650	188	6.20
4 Months	149	4.1	107	5.1	1.00	21	0.45	65.0	40.0	760	191	4.30
6 Months	150	4.4	99	5.2	1.05	23	0.40	32.0	28.0	580	142	4.70
8 Months	147	4.5	106	5.0	1.10	21	0.42	35.0	21.0	740	172	4.30
12 Months	152	4.4	109	5.2	1.40	26	0.20	32.0	21.0	500	176	4.70

[1]q.n.s. = quantity not sufficient.

Note: There was a trace of hemolysis at one and at two months.

TABLE 14. CLINICAL CHEMISTRY PROFILE OF ANIMAL NUMBER 1673 (MALE, EXPOSED)

	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	BILI. (mg%)	SGOT (U/ml)	SGPT (U/ml)	LDH (Sigma U)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	143	5.4	101	5.7	0.70	12	q.n.s.[1]	66.0	83.0	1230	163	3.60
Baseline II	142	5.1	98	5.3	0.80	15	0.2*	42.0	33.0	340	155	-
1 Month	139	3.7	100	5.2	0.70	15	0.35	35.0	38.0	390	175	-
2 Months	135	3.7	103	4.9	0.85	16	0.55	31.0	37.0	500	170	5.40
4 Months	144	5.2	102	5.4	0.80	16	0.60	30.0	40.0	570	190	4.70
6 Months	148	4.6	95	5.5	0.90	16	0.65	47.0	22.0	1170	185	5.70
8 Months	137	4.3	103	5.0	1.00	16	0.55	50.0	43.0	1220	169	4.70
12 Months	150	5.0	105	5.3	1.20	21	0.35	34.0	32.0	590	152	4.80

[1]q.n.s. = quantity not sufficient.

Note: There was a trace of hemolysis at one month only.

TABLE 15. CLINICAL CHEMISTRY PROFILE OF ANIMAL NUMBER 1671 (MALE, EXPOSED)

	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	BILI. (mg%)	SGOT (U/ml)	SGPT (U/ml)	LDH (Sigma U)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	172	7.9	109	4.3	1.9	10	0.07	72.0	27.1	1277	167	2.80
Baseline II	142	4.6	99	5.1	1.10	12	0.20	43.0	26.0	900	136	-
1 Month	140	4.3	101	5.1	0.90	13	0.30	51.0	13.0	1260	193	-
2 Months	142	4.6	104	5.1	0.95	13	0.45	34.0	16.0	500	190	5.50
4 Months	147	5.4	108	5.3	0.80	14	0.40	29.0	16.0	410	209	4.70
6 Months	144	4.6	98	5.6	1.70	16	0.42	22.0		1000	150	5.30
8 Months	141	4.3	102	5.0	1.70	17	0.40	72.0	40.0	1360	186	5.60
12 Months	146	4.6	103	5.4	1.40	20	0.20	44.0	18.0	1120	143	5.60

Note: There was no evidence of hemolysis.

TABLE 16. CLINICAL CHEMISTRY PROFILE OF ANIMAL NUMBER 1917 (FEMALE, EXPOSED)

	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	Bilir. (mg%)	SGOT (U/ml)	SGPT (U/ml)	LDH (Sigma U)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	150	6.6	107	5.7	0.95	10	0.10	54.0	25.0	900	153	3.30
Baseline II	144	4.2	97	5.3	0.80	11	0.05	40.0	23.0	670	145	-
1 Month	135	4.3	104	5.1	0.80	13	0.40	32.0	8.0	935	129	-
2 Months	142	4.5	108	4.9	0.70	13	0.40	31.0	19.0	560	130	3.70
4 Months	142	5.2	101	5.2	0.90	21	0.55	25.0	16.0	700	200	4.20
6 Months	143	5.1	90	5.4	0.80	16	0.50	39.0	22.0	1040	142	5.40
8 Months	142	4.7	107	4.8	0.80	16	0.50	45.0	18.0	1220	135	5.00
12 Months	149	4.2	107	4.9	1.10	27	0.10	40.0	18.0	1294	125	4.40

Note: There was no evidence of hemolysis.

TABLE 11. CLINICAL CHEMISTRY PROFILES OF NORMAL CHICKENS AND TURKEYS (CONTINUED)

Species	Age	Sex	Ca^{++}	Cl^-	K^+	Na^+	Urea Nitrogen (BUN)	Uric Acid	Total Protein (TP)	Albumin (Alb)	Globulin (Glob)	Alb/Glob Ratio	Glucose	Total Lipids (TL)	Cholesterol (Chol)	Triglycerides (Trig)	Phospholipids (PL)	Chol/PL Ratio	Alkaline Phosphatase (ALP)	Lactic Dehydrogenase (LDH)	Glutamic Oxaloacetic Transaminase (GOT)	Glutamic Pyruvic Transaminase (GPT)	Creatine Phosphokinase (CPK)	Aldolase	Reference
Broiler	1	M	9.5	102	4.3	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	11	M	9.6	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	21	M	9.7	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
Broiler	1	F	9.5	102	4.3	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	11	F	9.6	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	21	F	9.7	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
Broiler	1	M	9.5	102	4.3	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	11	M	9.6	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	21	M	9.7	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
Broiler	1	F	9.5	102	4.3	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	11	F	9.6	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	21	F	9.7	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
Broiler	1	M	9.5	102	4.3	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	11	M	9.6	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	21	M	9.7	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
Broiler	1	F	9.5	102	4.3	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	11	F	9.6	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	21	F	9.7	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
Broiler	1	M	9.5	102	4.3	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	11	M	9.6	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	21	M	9.7	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
Broiler	1	F	9.5	102	4.3	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	11	F	9.6	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1
	21	F	9.7	102	4.2	151	1.7	0.5	4.7	2.7	2.0	1.4	230	0.7	0.7	0.3	0.3	2.3	1,000	1,000	250	25	1,000	10	1

Note: These values are based on analysis of samples from [illegible]

TABLE 18. CLINICAL CHEMISTRY PROFILE OF ANIMAL NUMBER 1920 (FEMALE, EXPOSED)

	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	Bilir. (mg%)	SGOT (U/ml)	SGPT (U/ml)	LDH (Sigma U)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	180*	7.2	106	6.8	1.15	10	0.30	24.0	19.0	395	163	4.30
Baseline II	149	5.8	107	5.0	1.00	10	0.75	25.0	18.0	470	164	-
1 Month	139	4.1	104	4.7	0.80	14	0.35	34.0	16.0	810	209	-
2 Months	149	4.4	106	5.0	0.95	19	0.50	39.0	13.0	440	200	4.70
4 Months	146	5.5	108	5.0	0.95	17	0.46	23.0	14.0	410	191	3.60
6 Months	139	4.1	102	4.9	1.00	18	0.40	28.0	24.0	600	175	4.20
8 Months	148	4.5	108	4.8	1.20	14	0.40	24.0	14.0	470	170	3.80
12 Months	141	3.7	108	5.1	1.20	20	0.20	30.0	14.0	510	176	4.30

Note: There was a trace of hemolysis at one month and at 6.* months.

TABLE 19. CLINICAL CHEMISTRY PROFILE OF ANIMAL NUMBER 1502 (FEMALE, EXPOSED)

	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	Brili. (mg%)	SGOT (U/ml)	SGPT (U/ml)	LDH (Sigma U)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	149	6.6	104	5.7	1.20	14	0.15	40.0	27.0	700	200	3.80
Baseline II	144	5.0	101	5.1	0.90	17	0.05	25.0	17.0	530	164	-
1 Month	139	4.1	101	4.8	0.70	19	0.30	45.0	35.0	1150	184	-
2 Months	143	4.7	105	4.7	0.80	20	0.40	37.0	27.0	670	182	3.60
4 Months	148	5.2	106	5.0	0.90	25	0.48	36.0	34.0	640	200	3.60
6 Months	145	4.5	98	5.3	1.00	25	0.57	48.0	50.0	1020	192	4.80
8 Months	140	4.5	105	4.8	0.90	19	0.40	44.0	24.0	1220	170	2.90
12 Months	146	4.1	107	5.0	1.20	23	0.20	31.0	19.0	360	157	3.50

Note: There was a trace of hemolysis at two months only.

TABLE 20. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1403 (FEMALE, CONTROL)

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	12.2	36.0	5.78	10.9
Baseline II	12.0	36.0	-	-
1 Month	12.2	37.0	4.97	8.8
2 Months	13.0	37.0	4.91	9.4
4 Months	13.2	41.0	5.35	8.6
6 Months	12.2	36.0	4.77	6.8
8 Months	12.7	36.0	4.80	8.0
12 Months	12.7	36.0	4.84	6.9

TABLE 21. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1547 (FEMALE, CONTROL)

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	15.0	44.0	5.61	8.4
Baseline II	13.8	41.0	5.24	7.6
1 Month	14.4	43.0	5.38	14.2
2 Months	15.2	44.0	5.47	12.2
4 Months	14.4	43.0	5.66	12.8
6 Months	14.0	42.0	5.32	14.1
8 Months	14.4	42.0	5.53	14.2
12 Months	15.9	47.0	6.01	8.8

TABLE 22. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1704 (MALE, CONTROL)

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	15.2	44.0	5.77	8.4
Baseline II	14.0	42.0	4.91	7.7
1 Month	14.4	44.0	5.71	9.3
2 Months	16.0	48.0	6.18	9.6
4 Months	15.6	48.0	6.10	8.7
6 Months	15.2	46.0	6.02	5.4
8 Months	15.2	43.0	5.85	7.7
12 Months	13.4	40.0	5.29	9.2

TABLE 23. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1706 (MALE, CONTROL)

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	16.0	45.0	5.89	13.3
Baseline II	15.0	44.0	5.09	11.7
1 Month	14.4	42.0	5.23	9.5
2 Months	14.0	42.0	5.60	10.7
4 Months	15.2	46.0	5.52	10.6
6 Months	15.0	43.0	5.31	8.3
8 Months	15.7	43.0	5.71	9.3
12 Months	15.4	45.0	5.54	12.1

TABLE 24. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1867 (MALE, CONTROL)

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	14.0	43.0	5.35	17.1
Baseline II				
1 Month	13.8	40.0	5.20	11.1
2 Months	13.8	42.0	5.16	11.7
4 Months	13.2	41.0	5.42	12.3
6 Months	13.8	42.0	5.30	14.4

TABLE 25. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1916 (FEMALE, CONTROL)

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	16.0	52.0	6.18	10.7
Baseline II	15.2	46.0	5.18	7.8
1 Month	16.0	48.0	6.35	8.0
2 Months	13.8	43.0	5.37	7.4
4 Months	15.0	45.0	6.04	6.0
6 Months	14.0	46.0	5.74	7.4
8 Months	14.6	42.0	5.49	8.4
12 Months	15.4	45.0	5.91	8.0

TABLE 26. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1549 (MALE; EXPOSED)

	Hgb (gms)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	15.2	44.0	5.95	7.5
Baseline II	13.2	40.0	5.12	7.3
1 Month	15.6	45.0	5.50	6.9
2 Months	15.0	45.0	5.60	7.1
4 Months	15.0	42.0	5.00	6.8
6 Months	14.4	45.5	5.24	6.5

TABLE 27. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1869 (MALE, EXPOSED)

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	15.0	44.0	5.19	21.3
Baseline II	13.2	44.0	4.87	11.8
1 Month	15.0	44.0	5.96	8.2
2 Months	14.4	43.0	5.32	11.2
4 Months	15.1	44.0	5.80	9.9
6 Months	15.0	46.0	5.89	9.1
8 Months	15.0	42.0	5.85	7.9
12 Months	15.4	45.0	5.80	6.5

TABLE 28. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1870 (MALE, EXPOSED)

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	16.0	49.0	6.06	15.4
Baseline II	16.4	49.0	5.67	11.7
1 Month	16.0	46.0	5.91	8.2
2 Months	15.0	44.0	5.50	8.6
4 Months	16.0	48.0	5.89	7.6
6 Months	15.2	42.0	5.69	8.7
8 Months	16.6	48.0	6.20	8.1
12 Months	15.4	42.0	5.42	16.7

TABLE 29. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1071 (MALE, EXPOSED)

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	15.6	46.0	5.69	14.1
Baseline II	16.0	45.0	5.67	9.1
1 Month	16.0	45.0	5.83	10.3
2 Months	15.0	45.0	5.80	8.3
4 Months	14.4	43.0	5.05	8.6
6 Months	15.6	46.0	6.02	11.4
9 Months	16.4	45.0	5.83	11.7
12 Months	14.5	43.0	5.65	12.5

TABLE 30. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1917 (FEMALE, EXPOSED)

	Hgb (gm%)	PCV (vol %)	RBC (x 10^6)	WBC (x 10^3)
Baseline I	16.4	49.0	5.96	8.6
Baseline II	15.6	45.0	5.37	6.9
1 Month	16.4	47.0	6.23	9.7
2 Months	16.0	48.0	5.96	6.1
4 Months	15.2	46.0	5.69	8.4
6 Months	18.4	58.0	7.03	9.2
8 Months	16.8	46.0	6.04	8.9
12 Months	16.4	47.0	6.10	9.1

TABLE 31. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1919 (FEMALE, EXPOSED)

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	16.0	49.0	6.96	14.7
Baseline II	15.2	43.0	5.08	9.4
1 Month	15.2	44.0	5.70	17.4
2 Months	15.2	45.0	5.61	9.3
4 Months	15.2	45.0	5.83	8.1
6 Months	15.0	46.0	6.09	10.5
8 Months	15.2	43.0	5.87	7.2
12 Months	15.9	45.0	5.81	8.5

TABLE 32. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1920 (FEMALE, EXPOSED)

	Hgb (gms)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	14.4	42.0	4.84	7.0
Baseline II	15.0	44.0	5.81	5.9
1 Month	15.0	44.0	5.81	5.9
2 Months	15.2	44.0	5.40	7.2
4 Months	15.2	46.0	5.81	7.2
6 Months	15.2	45.5	5.32	6.4
8 Months	15.2	42.0	5.66	8.2
12 Months	15.4	46.0	5.87	7.2

TABLE 33. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1922 (FEMALE, EXPOSED)

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	16.0	44.0	6.54	18.7
Baseline II	15.0	43.0	5.01	9.0
1 Month	14.6	42.0	5.23	9.4
2 Months	15.0	45.0	5.48	9.8
4 Months	15.0	44.0	5.42	6.7
6 Months	15.0	42.0	5.38	6.7
8 Months	14.4	40.0	5.19	6.0
12 Months	14.8	43.0	5.50	10.3

TABLE 34. CONCENTRATION OF MANGANESE IN URINE OF MONKEYS EXPOSED TO AIRBORNE MANGANESE*

Animal	Baseline		Month of exposures				
Number	1	2	1	2	4	8	12
CONTROL							
1704M	12.2	14.6	9.1	7.0	13.7	7.2	< 1.0
1867M	8.0	3.8	5.5	22.3	15.8	- [1]	-
1547F	15.2	32.6	30.9	40.9	11.2	16.1	< 1.0
1916F	7.9	4.7	3.2	9.3	3.5	8.7	< 1.0
Mean	10.8	13.9	12.2	19.8	11.0	8.7	< 1.0
EXPOSED							
1549M	7.6	8.5	27.6	10.7	39.3	- [1]	-
1869M	4.0	2.4	17.0	10.6	28.9	3.0	10.2
1870M	4.1	7.7	15.8	14.6	18.0	2.0	5.1
1871M	9.4	8.9	21.4	15.6	68.4	29.6	68.0
1917F	16.7	15.8	70.0	20.6	52.2	4.5	3.7
1919F	6.7	4.8	39.3	12.9	29.0	8.4	12.1
1920F	12.6	5.3	30.6	21.0	45.6	14.3	14.9
1922F	5.0	1.0	12.8	9.9	33.2	1.5	77.0
Mean	8.3	6.8	29.3	14.5	39.3	9.0	27.3

*Data are expressed in µg Mn excreted per 24 hours

[1]Sacrificed after 6 months

TABLE 35. MANGANESE IN FECES OF MONKEYS EXPOSED TO AIRBORNE MANGANESE*

Animal	Baseline		Month of exposures				
Number	1	2	1	2	4	8	12
CONTROL							
1704M	1.67	2.91	4.20	5.92	4.93	4.83	7.29
1867M	2.62	1.92	2.15	-[2]	1.66	-[1]	
1547F	2.32	2.90	1.59	5.22	4.47	-[2]	5.72
1916F	2.51	2.65	4.31	10.52	4.66	9.36	2.88
Mean	2.31	2.59	3.06	7.22	3.93	7.10	5.30
EXPOSED							
1549M	4.39	-	1.20	1.57	1.08	-[1]	
1869M	1.03	3.48	1.66	0.91	0.33	3.87	0.71
1870M	1.06	3.96	0.38	0.32	1.29	-[2]	0.93
1871M	1.94	-	2.61	1.05	0.69	8.03	2.41
1917F	0.89	0.68	3.43	3e.48	1.34	3.07	2.29
1919F	4.55	2.89	3.53	1.58	2.35	2.18	2.47
1920F	2.82	5.06	2.61	1.96	1.91	1.12	2.16
1922F	1.22	0.44	1.08	1.42	1.61	1.92	1.58
Mean	2.21	2.75	2.06	5.16	1.32	3.36	1.79

*Data are expressed in milligrams of Mn excreted per 24 hours.

[1]Animal sacrificed after 6 months.

[2]Sample lost.

TABLE 36. MANGANESE IN SELECTED TISSUES OF MONKEYS EXPOSED TO AIRBORNE MANGANESE*

Animal Number	Lung	Liver	Pancreas	Spleen	Kidney
Control					
1867	0.42	2.40	1.51	0.39	0.82
1479	0.14	2.10	1.56	0.41	1.00
1731	0.12	0.74	1.39	0.21	1.05
1766	0.10	0.74	1.40	0.18	0.91
1781	0.09	1.31	1.18	0.16	0.80
Mean	0.17	1.46	1.41	0.27	0.92
S.D.	±0.14	±0.77	±0.15	±0.12	±0.11
Exposed 6 months					
1949	0.77	2.87	2.51	0.48	1.82
Exposed 12 months					
1870	0.39	3.00	2.12	0.37	2.00
1871	0.59	2.25	2.32	0.52	2.00
1917	1.21	2.35	2.22	0.36	1.93
1922	1.10	2.76	2.93	0.43	1.70
Mean	0.82**	2.46§	2.41**	0.42	1.91**
S.D.	±0.39	±0.36	±0.35	±0.07	±0.14
Exposed 15 months					
1869	0.26	1.75	2.02	0.27	1.16
1919	0.56	1.25	1.29	0.41	1.08
Mean	0.41	1.50	1.66	0.59	1.12

* Data are expressed as μg/g of wet tissue.

** Mean significantly different from control at 0.01 level of probability.

§ Mean significantly different from control at 0.05 level of probability.

(continued)

TABLE 36. (continued)

Animal number	Bone (Femur)	Adrenal	Heart muscle	Skeletal muscle
Control				
1867	0.12	2.00		
1479	0.60	1.78	0.48	0.16
1731	0.62	1.02	0.39	0.17
1765	0.92	2.16	0.27	0.13
1781	0.68	1.20	0.24	0.30
Mean	0.57	1.63	0.35	0.19
S.D.	±0.26	±0.50	±0.11	±0.07
Exposed 6 months				
1549	0.13	1.20		
Exposed 12 months				
1870	0.38	2.43	0.64	0.24
1871	0.11	3.04	0.63	0.47
1917	0.35	2.11	0.64	0.54
1922	0.34	1.60	0.64	0.28
Mean	0.29	2.30	0.64**	0.38
S.D.	±0.12	±0.60	±0.01	±0.15
Exposed 15 months				
1869	0.39	4.43	0.52	0.28
1619	0.47	3.37	0.61	0.25
Mean	0.43	3.90	0.56	0.26

** Mean significantly different from control at 0.01 level of probability.

TABLE 37. MANGANESE IN TISSUES FROM NERVOUS SYSTEM OF MONKEYS EXPOSED TO AIRBORNE MANGANESE*

Animal number	Pallium	Basal ganglia	Cerebellum	Pons	Medulla
Control					
1867	0.50	0.60	0.77	0.31	0.79
1479	0.61	0.50	0.44	0.10	0.39
1731	0.41	0.44	0.55	0.10	0.13
1765	0.37	0.41	0.50	0.17	0.17
1781	0.45	0.54	0.57	[illegible]	1.43
Mean	0.47	0.50	0.57	0.17	0.58
S.D.	±0.09	±0.08	±0.12	±0.10	±0.54
Exposed 6 months					
1549	0.80	1.00	0.96	0.44	0.96
Exposed 12 months					
1870	0.83	1.15	0.94	0.81	0.80
1871	0.63	1.08	0.96	0.72	0.53
1917	0.90	1.11	1.29	0.89	0.89
1922	0.94	1.67	1.04	0.79	1.92
Mean	0.83**	1.25**	1.06**	0.80**	1.04
S.D.	±0.14	±0.28	±0.16	±0.07	±0.61
Exposed 15 months					
1869	0.37	1.29	0.93	1.34	0.86
1919	0.44	0.93	1.03	0.52	0.68
Mean	0.41	1.11	0.99	0.93	0.77

* Data are expressed as a μg/g of wet tissue.

** Mean significantly different from controls at 0.01 level of probability.

TABLE 38. EXCRETION OF MANGANESE IN URINE OF RATS EXPOSED TO AIRBORNE MANGANESE

Exposure condition	Animal number	Concentration ug/ml	24 Hour excretion, ug
Pre-exposure	1	0.04	0.32
	2	0.09	0.20
	3	0.11	0.61
	4	0.06	0.39
	Mean ± S.D.	0.07 ± 0.03	0.38 ± 0.17
1 Week	1	0.10	0.90
	2	0.15	1.52
	3	0.35	5.43
	4	0.13	1.25
	Mean ± S.D.	0.18 ± 0.11	2.27 ± 2.12
4 Weeks	1	0.09	0.90
	2	0.09	1.26
	3	0.15	2.68
	4	0.07	0.98
	Mean ± S.D.	0.10 ± 0.03	1.45 ± 0.83
8 Weeks	1	0.07	0.98
	2	0.40	0.96
	3	0.10	1.00
	4	0.04	0.44
	Mean ± S.D.	0.17 ± 0.20	0.84 ± 0.27
1 Week post-exp.	1	0.10	2.10
	2	0.04	1.04
	3	0.37	5.90
	4	0.09	0.90
	Mean ± S.D.	0.15 ± 0.15	2.48 ± 2.34
4 Weeks post-exp.	1	0.11	2.64
	2	0.40	7.20
	3	0.20	1.60
	4	0.14	2.52
	Mean ± S.D.	0.21 ± 0.13	3.49 ± 2.62
8 Weeks post-exp.	1	0.08	0.67
	2	0.08	0.57
	3	0.12	0.65
	4	0.15	0.83
	Mean ± S.D.	0.11 ± 0.03	0.73 ± 0.13

TABLE 19. EXCRETION OF MANGANESE IN FECES OF RATS EXPOSED TO AIRBORNE MANGANESE

Exposure condition	Animal number	Concentration mg/g	24 Hour excretion, mg
Pre-exposure	1	0.66	1.06
	2	0.36	0.48
	3	0.46	0.94
	4	0.43	0.18
	Mean ± S.D.	0.48 ± 0.13	0.66 ± 0.41
1 Week	1	0.65	2.97
	2	0.64	2.78
	3	0.75	5.29
	4	0.70	2.57
	Mean ± S.D.	0.68 ± 0.09	3.40 ± 1.26
4 Weeks	1	0.48	2.45
	2	0.56	3.10
	3	0.62	1.82
	4	0.43	1.88
	Mean ± S.D.	0.52 ± 0.08	2.31 ± 0.60
8 Weeks	1	0.60	2.99
	2	0.80	5.60
	3	0.61	3.06
	4	0.71	3.06
	Mean ± S.D.	0.68 ± 0.10	3.67 ± 1.28
1 Week post-exp.	1	0.57	2.83
	2	0.61	11.56
	3	0.55	3.31
	4	0.56	2.02
	Mean ± S.D.	0.57 ± 0.03	6.01 ± 4.37
4 Weeks post-exp.	1	0.37	1.13
	2	0.33	0.91
	3	0.35	0.28
	4	0.35	0.05
	Mean ± S.D.	0.36 ± 0.02	0.79 ± 0.36
8 Weeks post-exp.	1	0.93	6.05
	2	0.98	4.41
	3	1.08	4.01
	4	1.12	4.94
	Mean ± S.D.	1.03 ± 0.08	5.05 ± 0.70

TABLE 40. MANGANESE IN TISSUES OF RATS EXPOSED TO AIRBORNE MANGANESE*

Exposure condition	Animal sex	Liver	Kidney	Lung	Brain
Pre-exposure	M	1.02	0.63	0.36	0.54
	F	1.76	0.82	0.27	0.59
1 Week	M	1.61	0.79	0.58	0.67
	F	1.80	1.53	0.96	0.86
4 Weeks	M	1.46	0.74	0.73	0.68
	F	1.65	0.67	0.29	0.83
8 Weeks	M	2.16	1.06	0.97	0.97
	F	1.82	0.71	0.97	0.83
1 Week post-exp.	M	1.61	0.82	0.30	0.50
	F	1.39	0.62	0.21	0.44
4 Weeks post-exp.	M	1.45	0.65	0.31	0.74
	F	1.83	0.83	0.46	0.93
8 Weeks post-exp.	M	1.32	0.71	0.47	0.60
	F	1.87	0.64	0.30	0.64

* Data are expressed in μg Mn/g of wet tissue.

TABLE 41. WEEKLY MEAN LEVELS OF MANGANESE IN AIR OF HIGH-LEVEL EXPOSURE CHAMBERS*

Week	Chamber A	Chamber B
1	2620	3240
2	2900	4630
3	1630	1760
4	1380	1480
5	2440	1450
6	2100	1830
7	2460	2120
8	3860	3840
9	4670	3880
10	4300	5040
11	5890	7250
12	12940	11590
13	9160	11340
14	6380	6060
15	6900	5910
16	6900	6820
17	7380	6930
18	6450	5610
19	3940	3670
20	3170	3280
21	3460	3230
22	7290	6460
23	5940	6430
Mean	4860	5100

*Data are expressed as μg Mn/m³ of air.

TABLE 42. CLINICAL CHEMISTRY PROFILE OF ARCHIVAL NUMBER TEST*

	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	Bilir. (mg%)	SGOT (U/ml)	SGPT (U/ml)	LDH (Sigma U)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	138	4.4	103	4.8	1.2	32	0.50	46	48	1320	200	6.9
Baseline II	142	4.5	105	4.8	0.8	20	0.50	31	14	520	177	4.5
1 Month	145	4.6	104	5.4	1.2	17	0.40	14	13	168	178	8.2
2 Months	147	4.6	112	5.0	1.7	25	0.44	22	18	184	150	8.6
3 Months	150	5.5	101	5.1	1.3	25	0.18	13	11	125	150	-
4 Months	146	5.0	115	5.5	1.5	16	0.14	20	13	560	200	7.4
5 Months	151	5.3	109	4.7	1.5	20	0.58	25	20	201	171	2.9

* Rhesus monkey (male, exposed)

TABLE 43. CLINICAL CHEMISTRY PROFILE OF ANIMAL NUMBER 1654*

	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)	Ca^{++} (mEq/l)	Creat. (mg%)	BUN (mg%)	Bilir. (mg%)	SGOT (u/ml)	SGPT (u/ml)	LDH (Sigma u)	Chol. (mg%)	PO_4^{-3} (mg/dl)
Baseline I	143	3.8	.08	5.0	1.0	42	0.38	25	18	400	140	4.3
Baseline II	144	4.5	107	5.2	0.8	12	0.40	25	14	440	154	5.1
1 Month	147	4.4	106	5.3	1.2	12	0.20	11	14	102	133	7.2
2 Months	148	4.4	114	5.1	1.4	19	0.42	18	18	240	125	8.6
3 Months	144	4.3	105	5.8	1.1	16	0.03	16	16	99	-	-
4 Months	146	4.2	112	5.5	1.1	13	0.70	27	14	353	130	5.9
5 Months	149	4.8	110	4.7	1.0	12	0.42	16	21	125	162	9.8

* Rhesus monkey (male, exposed)

TABLE 44. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1001*

	Hgb (gm%)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	13.6	39.0	4.29	5.9
Baseline II	12.7	35.0	4.96	8.4
1 Month	15.4	46.0	5.91	5.7
2 Months	14.0	42.0	5.49	4.0
3 Months	-	-	-	-
4 Months	18.4	42.0	5.43	9.4
5 Months	15.9	48.0	5.96	8.7

* Rhesus monkey (male, exposed)

TABLE 46. HEMATOLOGY PROFILE OF ANIMAL NUMBER 1884*

	Hgb (gms)	PCV (vol %)	RBC ($\times 10^6$)	WBC ($\times 10^3$)
Baseline I	14.4	42.0	4.45	8.1
Baseline II	14.2	41.0	5.54	8.1
1 Month	17.5	54.0	6.63	9.2
2 Months	14.7	48.0	6.29	5.9
3 Months	12.9	41.0	5.43	13.0
4 Months	16.0	46.9	5.94	12.3
5 Months	15.5	47.0	5.55	10.7

* Rhesus monkey (male, exposed)

TABLE 46. EXCRETION OF MANGANESE BY MONKEYS EXPOSED TO AIRBORNE MANGANESE*

	Monkey #1001		Monkey #1004	
	Urine ugMn/24hr	Feces ug/Mn/g	Urine ugMn/24hr	Feces ug/Mn/g
Baseline				
1	17	200	9	275
2	40	215	15	285
Exposed				
1 Month	246	400	426	465
2 Months	249	650	193	585
3 Months	198	415	323	555
4 Months	109	400	657	450
5 Months	310	365	465	510

* Exposure at about 5000 ug/m^3

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PATHOLOGY APPENDIX
TO THE CHRONIC INHALATION STUDY
ON AIRBORNE PARTICULATE MANGANESE BY RHESUS MONKEYS

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CONTENTS OF PATHOLOGY APPENDIX

Figures 70
Tables 71

A. Material and Methods 72

B. Results 72

Body and Organ Weights 72
Organ Observations 73
Microscopic Observations 74

C. Discussion 76

D. Conclusions 77

FIGURES

Page

Figure A-1 78

Figure A-2 79

Figure A-3 80

Figure A-4 81

TABLES

	Page
Table A-1	82
Table A-2	83
Table A-3	84

PATHOLOGY APPENDIX

A. MATERIAL AND METHODS

A total of twelve male and female rhesus monkeys were used in the present study (Table A-1).

Complete necropsies were performed on all animals with special attention given to the lips, tongue, oral cavity, external ears, eyes, limbs, vagina, and anus. Each organ was examined *in situ* and after removal under low power (10x) magnification.

Representative samples from salivary glands, mammary tissue, liver, stomach, duodenum, jejunum, colon, pancreas, adrenals, kidneys, urinary bladder, mesenteric lymph nodes, esophagus, trachea, lungs, heart, thymus, thyroids, parathyroids, uterus and tongue were fixed in 10% buffered formalin. The eyes were fixed in Zenker's acetic acid solution, the ovaries in Bouin's solution, and the central nervous system in 37 - 40% buffered formalin. Calcified structures were decalcified in a mixture of 45% aqueous formic acid and a 20% aqueous solution of Na citrate 1/1 (v/v).

Regions of the central nervous system were sampled at the following levels: The first frontal section was through the precentral gyrus, the second through the Ammon's horn, and the third through the calcarine sulcus; the first transverse section was through the mesencephalon, the second through the pons and part of cerebellum, the third through the medulla and part of the cerebellum and the fourth through the cervical spinal cord.

All sampled tissues were routinely dehydrated and embedded in paraffin. Sections were cut at 6µ and stained with hematoxylin (Harris's) and eosin - (Eosin Y). One kidney was also stained with periodic acid-Schiff technique (PAS). Body weights and organ weights from liver, kidney and brain (including cerebellum, pons and medulla) were recorded and the relative organ weights calculated.

B. RESULTS

1. Body and Organ Weights

Final body weights, absolute and relative organ weights, obtained at necropsy are listed in Table A-2.

J: Gross Observations on Rhesus Monkeys

No. 1867, male (control) 6 months:

Mesentric lymph nodes (MLN) = Extensive enlargement, upon cutting, small cystic structures became visible.
The rest of organs and systems = No other pathologic changes evident.

No. 1549, Male (100 μg/m³) 6 months:

Lungs = Mites were present bilaterally.
The rest of organs and systems = No other pathologic change was evident.

No. 1479, female (control) 12 months:

No pathologic change was evident in any of the organs and systems.

No. 1731, female (control) 12 months:

Lungs = changes consistent with pneumonia were present bilaterally.
Kidneys = Both were pale.
The rest of organs and systems = No pathologic change was evident.

No. 1765, male (control) 12 months:

Lungs = changes consistent with pneumonia were present bilaterally.
The rest of organs and systems = No other pathologic change was evident.

No. 1781, male (control) 12 months:

Spleen = Granular.
The rest of organs and systems = No other pathologic change was evident.

No. 1870, male (100 μg/m³) 12 months:

Lungs = Mites were present bilaterally.
The rest of organs and systems = No other pathologic change was evident.

No. 1871, male (100 μg/m³) 12 months:

Lungs = Mites were present bilaterally.
The rest of organs and systems = No other pathologic change was evident.

No. 1917, female (100 μg/m³) 12 months:

Lungs = Mites were present bilaterally.
Abdominal cavity = The lower pole of the left kidney was adhered to the lateral peritoneum where a parasitic abscess was visible.
The rest of organs and systems = No other pathologic change was evident.

No. 1822, female (100 μg/m³) 12 months:

Lungs = Mites were present bilaterally.
The rest of organs and systems = No other pathologic change was evident.

No. 1869, male (100 μg/m³) 15.5 months:

Lungs = Mites were present bilaterally.
The rest of organs and systems = No other pathologic change was evident.

No. 1919, female (100 μg/m³) 15.5 months:

No pathologic change was evident in any of the organs and systems.

3. Microscopic Observations

A summary of the microscopic changes is given on Table A-1. Below, only the organs and tissues with pathologic changes will be listed in detail, the rest will be understood to have been within normal limits.

No. 1867, male (control) 6 months:

Liver = Few, focal single cell necroses of mild intensity.
Kidney = Slight, occasional, interstitial mononuclear infiltrates.
Lung = Mild form of acariasis with peribronchiolitis and pneumonitis and a tan to yellow-brown granular pigment within the macrophages.
NLN = Multiple parasitic abscesses with giant cells and pigment.

No. 1949, male (100 μg/m³) 6 months:

Liver = Few, focal, single cell necroses of mild intensity; mild lymphocytic and monocytic infiltration in one periportal field.
Lung = Mild form of acariasis with peribronchiolitis and pneumonitis and a tan to yellow-brown granular pigment within the macrophages.

No. 1979, female (control) 12 months:

Liver = Mild centrolobular hepatocellular hypertrophy with fine vacuolation; few, focal, single cell necroses of mild intensity with few mononuclear infiltrates.
Lung = Mild form of acariasis with peribronchiolitis and pneumonitis and a tan to yellow-brown granular pigment with the macrophages.
Spleen = Congestion and lymphoid depletion.

No. 1731, female (control) 12 months:

Liver = Mild centrolobular hepatocellular hypertrophy with fine vacuolation; few focal, single cell necroses of mild intensity with few mononuclear infiltrates.
Lung = Edema, atelectasis and peribronchiolitis.

No. 1765, male (control) 12 months:

Liver - Mild centrolobular hepatocellular hypertrophy with fine vacuolation; few, focal, single cell necroses of mild intensity with new mononuclear infiltrates.
Lung - Edema, atelectasis and peribronchiolitis.
Spleen - Congestion and lymphoid depletion.

No. 1781, male (control) 12 months:

Kidney - Slight, occasional, interstitial mononuclear infiltrates.
Liver - Mild centrolobular hepatocellular hypertrophy with fine vacuolation; few, focal, single cell necroses of mild intensity with few mononuclear infiltrates.
Lung - Congestion with focal areas of atelectasis.
Spleen - Slight reactive hyperplasia.

No. 1870, male (100 ug/m^3) 12 months:

Liver - Centrolobular distension of sinusoids.
Lung - Extensive form of acariasis with atelectasis, peribronchiolitis, pneumonitis, multinucleated giant cells, abundant eosinophils and a tan to yellow-brown granular pigment within the macrophages.

No. 1871, male (100 ug/m^3) 12 months:

Liver - Mild centrolobular hepatocellular hypertrophy with fine vacuolation.
Lung - Extensive form of acariasis with atelectasis, peribronchiolitis, pneumonitis, giant cells and a tan to yellow-brown granular pigment within the macrophages.
Heart - Focal area of infiltration consisting of monocytes and small and large lymphocytes; focal necrosis of muscle fibers (Fig. A-1).

No. 1917, female (100 ug/m^3) 12 months:

Liver - Centrolbular distension of sinusoids; few, focal, single cell necroses of mild intensity.
Kidney - Few, focal, mild, interstitial infiltrates.
Lung - Severe form of acariasis with atelectasis, peribronchiolitis, pneumonitis and a tan to yellow-brown granular pigment within the macrophages.
Nonencephalon - Behind the area of the colliculus commissure and within the gray matter, palisading was present; this consisted of proliferation of astrocytes in a concentrated fashion (Fig. A-2).

No. 1922, female (100 ug/m^3) 12 months:

Liver - Mild centrolobular hepatocellular hypertrophy with fine vacuolation; few, focal single cell necroses of moderate intensity with few mononuclear infiltrates.

No. 1922 (continued)

Lung - Moderate degree of acariasis with atelectasis, peribronchiolitis, pneumonitis and a tan to yellow-brown granular pigment within the macrophages.
Heart - Focal areas of infiltration consisting of monocytes and small and large lymphocytes; focal necrosis of muscle fibers. (Fig. A-3)
Internal capsule - Mild, focal, perivascular mononuclear cuffing.

No. 1869, male (100 $\mu g/m^3$) 15.5 months:

Liver - Mild centrolobular hepatocellular hypertrophy with fine vacuolation.
Kidney - Solid cortical adenoma; the lumens of the involved tubules were not patent; towards the center of these solid tubules a PAS positive material was present; focal areas of interstitial infiltration was present; focal areas of interstitial infiltration within the adenoma.
Lung - Moderate degree of acariasis with atelectasis, peribronchiolitis and a tan to yellow-brown granular pigment within the macrophages.

No. 1919, female (100 $\mu g/m^3$) 15.5 months:

Liver - Mild centrolobular hepatocellular hypertrophy with fine vacuolation.
Kidney - Slight, occasional, interstitial mononuclear infiltrates.
Heart - Focal areas of infiltration consisting of monocytes and small and large lymphocytes; focal necrosis of muscle fibers (Fig. A-4).
Muscle (rectus femoris) - Granuloma with multinucleated giant cells and eosinophils surrounding parasitic aldi.

C. DISCUSSION

The following changes which were observed constitute reactive changes to microbial agents (Table A-II): pulmonary acariasis, in eight out of twelve animals; parasitic granulomas and abscesses (2 out of 12); pulmonary atelectasis (3 out of 12); interstitial renal infiltrates (5 out of 12); splenic lymphoid depletion or reactive hyperplasia; and finally necroses in the liver (8 out of 12).

The mild centrolobular hepatocellular hypertrophy with the accompanying fine vacuolation (vacuolation), which were observed in both control and exposed monkeys, are probably responses to physiologic stimuli.

The astrocytic proliferation within the gray matter of the mesencephalon, which was observed in only one animal (No. 1917 C); and the perivascular cuffing within the internal capsule, which was observed in another animal (No. 1922 E), at the 12 month time intervals are probably coincidental. They were observed only in female monkeys at the 12 month interval, and not in animals of longer exposure. These are reactive changes; because of their location, are probably (but not conclusively) due to a transient anoxic anoxia.

The only growth observed was the renal adenoma in one male rhesus of the 15.5 month interval. This spontaneous tumor (renal tumor) is according to the literature (O'Gara and Adamson, 1972*) among the most frequent spontaneous growth of subhuman primates.

The minimal monocytic and lymphocytic infiltration of cardiac tissue, which was observed in two 12 month (No. 1871M and 1922F) and one 15.5 month rhesus monkeys, although it cannot be attributed to the inhalation of manganese at this time, deserves further investigation. Maybe a specific followup will be indicated.

D. CONCLUSIONS

1. No gross or microscopic changes were observed in any of the tissues or organs of rhesus monkeys attributable to inhalation of 100 $\mu g/m^3$ manganese for up to 15.5 months.

2. However, in three monkeys (No. 1871 M, 12 months; No. 1922 F, 12 months; and No. 1919 F, 15.5 months) out of seven monkeys (Table A-3) minimal monocytic and lymphocytic infiltration of cardiac tissue was observed. The significance of this observation cannot at this time be attributable to the inhalation of manganese.

* R.W. O'Gara and R. H. Adamson 1972. Spontaneous and Induced Neoplasms in Nonhuman Primates, in Pathology of Simian Primates Part I, pp. 215.

Figure A-1. Cardiac muscle from adult male rhesus (No. 1871), which received 100 $\mu g/m^3$ manganese for 12 months. Focal area of monocytic and lymphocytic infiltration. Hematoxylin and eosin, X376.

Figure A-8. Mesencephalon from adult female rhesus (No. 1917), which received 100 μg/m³ manganese for 12 months. In the area behind the sulcular commissure and within the white matter astrocytic proliferation is present. Hematoxylin and eosin, X372.

Figure A-3. Cardiac muscle from adult, female rhesus (No. 1922) which received 100 µg/m³ manganese for 12 months. Monocytic and lymphocytic infiltration and muscle fiber necroses are present (arrow). Hematoxylin and eosin, X364.

Figure A-4. Cardiac muscle from adult female rhesus (No. 1919), which received 100 ug/m³ manganese for 15.5 months. Focal areas of monocytic and lymphocytic infiltration are visible. Hematoxylin and eosin, X372.

TABLE 7-1 CONFIDENTIAL DATA ON THE CHEMICAL INDUSTRY STUDY OF ASTHMA

Name	Date	Time	Location	Notes
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE A-2. WEIGHTS* OF RHESUS MONKEYS EXPOSED TO CHRONIC INHALATION OF AIRBORNE PARTICULATE MANGANESE

Animal no. and sex	Body weight	Absolute liver weight	Relative liver weight	Absolute kidney weight	Relative kidney weight	Absolute brain weight	Relative brain weight
Control							
1427 F	3.70	104.0	2.67	8.50	0.23	89.0	2.40
1710 F	2.90	118.5	4.10	9.00	0.31	93.5	3.22
1765 F	2.50	107.0	4.28	6.00	0.24	78.0	3.12
1781 M	2.60	93.0	3.32	6.50	0.23	86.3	3.08
1867 M	3.80	77.6	1.98	8.35	0.22	98.0	2.58
Exposed 6 Months							
1546 M	6.30	103.0	1.62	9.00	0.14	90.0	1.43
Exposed 12 Months							
1870 M	5.20	84.0	1.61	8.50	0.16	92.0	1.77
1871 M	5.90	107.5	1.82	9.10	0.15	101.0	1.71
1917 F	5.80	83.5	1.44	9.50	0.16	80.0	1.40
1922 F	4.70	79.5	1.69	8.75	0.18	74.5	1.60
Exposed 15.5 Months							
1868 M	6.1	96.0	1.57	10.60	0.17	95.0	1.39
1919 F	6.7	119.0	1.77	10.80	0.16	91.0	1.35

* Body weight (kg), absolute (g), and relative (%) organ weights.

TABLE A-8. SUMMARY OF MICROSCOPIC CHANGES IN RHESUS MONKEYS EXPOSED TO CHRONIC* INHALATION OF AIRBORNE PARTICULATE MANGANESE

Group Identification	Organ	Change	Incidence**
Control, 6 months	Liver	Single cell necroses	1/1
	Lung	Acariasis	1/1
	Kidney	Interstitial infiltration	1/1
	MLN†	Parasitic abscesses	1/1
100 ug/m³, 6 months	Liver	Single cell necroses	1/1
		Periportal infiltration	1/1
	Lung	Acariasis	1/1
Control, 12 months	Liver	Single cell necroses	4/4
		Centrolobular hypertrophy and vacuolation	4/4
	Lung	Acariasis	1/4
		Atelectasis	3/4
	Kidney	Interstitial infiltration	1/4
	Spleen	Lymphoid depletion	2/4
		Reactive hyperplasia	1/4
100 ug/m³, 12 months	Liver	Single cell necroses	3/4
		Centrolobular hypertrophy and vacuolation	2/4
	Lung	Acariasis	4/4
	Kidney	Interstitial infiltration	1/4
	Heart	Single fiber necroses with infiltration	2/4
	Mesencephalon	Palisading	1/4
	Internal capsule	Perivascular cuffing	1/4
100 ug/m³, 15.5 months	Liver	Centrolobular hypertrophy and vacuolation	2/2
	Lung	Acariasis	1/2
	Kidney	Interstitial infiltration	1/2
		Adenoma	
	Heart	Single fiber necrosis with infiltration	1/2
	Muscle	Parasitic granuloma	1/2

* Chronic exposure was for 6 to 15.5 months

** Expressed in number of occurrence over the number of animals in the group

† Mesenteric lymph node